

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding a carotenoid biosynthetic pathway enzyme, selected from the group consisting of:
 - 5 (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12 and 14;
 - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
 - 10 (c) an isolated nucleic acid molecule that is complementary to (a) or (b).
2. The isolated nucleic acid molecule of Claim 1 selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11 and 13.
3. An isolated nucleic acid fragment of Claim 1 isolated from *Pantoea stewartii* strain DC413.
4. A polypeptide encoded by the isolated nucleic acid molecule of Claim 4.
- 20 5. The polypeptide of Claim 7 selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12 and 14.
6. An isolated nucleic acid molecule as set forth in SEQ ID NO:20, comprising the *crtE*, *idi*, *crtX*, *crtY*, *crtI*, *crtB*, and *crtZ*, genes or an isolated nucleic acid molecule having at least 95% identity to SEQ ID NO:20, wherein the isolated nucleic acid molecule encodes all of the polypeptides *crtE*, *idi*, *crtX*, *crtY*, *crtI*, *crtB*, and *crtZ*.
- 25 7. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a geranylgeranyl pyrophosphate synthetase enzyme of at least 302 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:2;
- 30 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
8. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an isopentenyl pyrophosphate isomerase enzyme of at least 344 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:4;
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or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

9. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an crtX enzyme of at least 429 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

10. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a lycopene cyclase enzyme of at least 387 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:8;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

11. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene desaturase enzyme of at least 493 amino acids that has at least 81% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:10;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

12. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene synthase enzyme of at least 309 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:12;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

13. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a β -carotene hydroxylase enzyme of at least 177 amino acids that has at least 82% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:14;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

14. A chimeric gene comprising the isolated nucleic acid molecule of any one of Claims 1 or 7-13 operably linked to suitable regulatory sequences.

5 15. A vector comprising the isolated nucleic acid molecule of Claim 6.

16. A transformed host cell comprising the chimeric gene of Claim 14.

17. A transformed host comprising the isolated nucleic acid molecule of claim 6.

10 18. The transformed host cell of Claim 16 or 17 wherein the host cell is selected from the group consisting of bacteria, yeast, filamentous fungi, algae, and green plants.

15 19. The transformed host cell of Claim 16 or 17 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*, *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*,
20 *Methylococcus*, *Methylosinus*, *Methylobacterium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, *Torulopsis*, *Phaffia*, and
25 *Rhodotorula*.

20. A method for the production of carotenoid compounds comprising:

- 30 (a) providing a transformed host cell comprising:
(i) suitable levels of farnesyl pyrophosphate; and
(ii) a set of nucleic acid molecules encoding the enzymes selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12 and 14 under the control of suitable regulatory sequences;
35 (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

21. A method for the production of carotenoid compounds comprising:

- (a) providing a transformed host cell comprising:
 - (i) suitable levels of farnesyl pyrophosphate; and
 - 5 (ii) a the isolated nucleic acid molecule of claim 6 under the control of suitable regulatory sequences;
- (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

10 22. A method according to Claim 20 or 21 wherein the transformed host cell is selected from the group consisting of C1 metabolizing hosts, bacteria, yeast, filamentous fungi, algae, and green plants.

23. A method according to Claim 22 wherein the C1 metabolizing host is a methanotroph and the fermentable carbon substrate is selected
15 from the group consisting of methane, methanol, formaldehyde, formic acid, methylated amines, methylated thiols, and carbon dioxide.

24. A method according to Claim 23 wherein the C1 metabolizing host:

- (a) grows on a C1 carbon substrate selected from the group
20 consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate-dependent phosphofructokinase enzyme.

25 25. A method according to Claim 24 wherein the C1 metabolizing host cell is a high growth methanotrophic bacterial strain, known as *Methylomonas* 16a and having the ATCC designation PTA 2402.

26. A method according to Claim 20 or 21 wherein the transformed host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*,
30 *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*,
35 *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Paracoccus*,

Nocardia, Arthrobacter, Rhodopseudomonas, Torulopsis, Phaffia, and Rhodotorula.

27. A method according to Claim 20 or 21, wherein the carotenoid compound produced is selected from the group consisting of
5 antheraxanthin, adonirubin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin, β -cryptoxanthin, α -carotene, β -carotene, epsilon-carotene, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, γ -carotene, 4-keto- γ -carotene, ζ -carotene, α -cryptoxanthin, deoxyflexixanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol,
10 isorenieratene, lactucaxanthin, lutein, lycopene, myxobactone, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, 4-keto-rubixanthin, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, 4-keto-torulene, 3-hydroxy-4-keto-torulene, uriolide, uriolide acetate, violaxanthin, zeaxanthin- β -
15 diglucoside, and zeaxanthin.

28. A method of regulating carotenoid biosynthesis in an organism comprising over-expressing at least one carotenoid biosynthetic pathway gene selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and 20 in an organism such that the carotenoid biosynthesis is altered
20 in the organism.

29. A method according to Claim 28 wherein said carotenoid gene is over-expressed on a multicopy plasmid.

30. A method according to Claim 28 wherein said carotenoid gene is operably linked to an inducible or regulated promoter.

25 31. A method according to Claim 28 wherein said carotenoid gene is expressed in antisense orientation.

32. A method according to Claim 28 wherein said carotenoid gene is disrupted by insertion of foreign DNA into the coding region.

30 33. A strain DC413 comprising the 16S rDNA sequence as set forth in SEQ ID NO:18.

34. An isolated nucleic acid molecule encoding all of the amino acid sequences as set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, and 14.

35 35. The isolated nucleic acid molecule of claim 40 having the nucleic acid sequence as set forth in SEQ ID NO:20.